MSCs and animal reproduction: use of adipose derived MSCs in an in vitro co-cultutre system produced bovine embryos

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Abstract

In vitro embryo production (IVEP) is a biotechnology used to multiply genetically superior animals in a short time interval. In bovines it is very common to cultivate fertilized embryos on top of a granulosa cell monolayer derived from the oocyte (called "co-culture") with the objective of reducing toxic metabolites and to protect embryos against oxidative stress. Adipose derived MSCs (AMSCs) are multipotent cells, secrete growth factors and cytokines, and can be easily obtained. This study aimed to compare bovine AMSCs (b-AMSCs) and granulosa cells in a co-culture of bovine IVEP. We hypothesized that b-AMSCs could replace efficiently granulosa cells in co-culture of bovine IVEP. Bovine-ADMSCs were isolated from fat of male adults male with collagenase type I (1µg/mL) and cultured in IMDM supplemented with 10% FBS and antibiotics. Stemness of b-ADMSCs was evaluated at passage 3 (p3) by immunophenotyping (CD73, CD90 and CD105) and in vitro differentiation (bone stained with 2% Alizarin Red S; adipose stained with 25% Oil Red; cartilage stained with Alcian Blue 1%) using STEMPro Kit. For IVEP, bovine oocytes were obtained in abattoir and in vitro matured in TCM-199 supplemented with 10% FBS, FSH and LH for 20 hours. Mature oocytes were fertilized with semen obtained from only one bull for 24h and the resulting embryos were cultivated in 100 µL droplets of SOF medium supplemented with 5% FBS, 6 mg/mL BSA for 7 days. IVEP was undertaken at 38.5 °C in 5% CO_2 incubators. Experimental groups were based on cell type used in the co-culture system: Graulosa cells (group GRAN) or b-ADMSCs (1,000 and 10,000 cells, in between passage p3-p6). A group of embryos were cultivated without cells (CTRL). Blastocyst rate was evaluated on the seventh day after fertilization and the total cell number per blastocysts was estimated by nuclear staining with DAPI (10µg/mL). There was an combined effect of cell passage and cell amount of b-ADMSCs on the IVEP outcome (Two-way ANOVA; $p<0.05$) with the group 1,000 b-ADMSCs at earlier passages (p3-p4) rendering better IVEP results in comparison to 10,000 b-ADMSCs unregardless of cell passage. When compared to CTRL and GRAN groups, the 1,000 b-ADMSCs (p3-p4) group showed higher blastocyst rate (33.53%, 36.07% vs. 48.49%, respectively; $p<0.05$) and total cell number per blastocyst (125.68±52.06, 148.18±42.54 vs. 173.11±50.88, respectively; $p<0.05$). Bovine-ADMSCs up

to passage 4 are better than granulosa cells when utilized in a co-culture system in bovine IVEP, increasing the amount of blastocysts obtained as well as the quality of them (measured in terms of total cell number)

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